

THE BOTANICAL GAZETTE

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EFFECT OF ANESTHETICS UPON RESPIRATION

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(WITH SEVEN FIGURES)

The special interest which this subject has acquired, as the result of certain modern theories, makes it desirable to give a brief review of some of the more important contributions (5, 10) to our knowledge of it.

MEYER (19) and OVERTON (21) independently concluded that the effect of a narcotic increases with its solubility in substances of a lipoid nature. According to them narcosis does not appear until the lipoids of the cells have absorbed the narcotic to a definite molecular concentration (21). The theory has been criticized (5, 10) because it fails to explain why narcotics, such as benzamide and monacetin, which at higher temperature are less soluble in fat, have an effect which increases with the temperature. Both MEYER and OVERTON recognize the fact that often there is no relation between the narcotic power of a substance and its relative solubility in oil; the partition quotient for isobutyl alcohol is about 180 times greater than for ethyl alcohol, but its power to cause narcosis is only about 6 times as great as that of ethyl alcohol. They state that such cases cannot be used as arguments against the lipoid theory in case the narcotic is not chemically indifferent, but has a special reaction affinity, as is the case, for example, with the basic narcotics.

VERWORN (29) has advocated the view that narcotics interfere with the oxygen carriers of the cell and render them incapable of

activating the molecular oxygen. As a result oxidation cannot take place and disintegration occurs, the cells thereby being asphyxiated. In this connection he makes the statement that a more or less complete recovery from narcosis, which may occur even in an oxygen-free medium, is at the cost of the oxygen contained within the living substance, which (on account of the suppression of the oxidation processes) could not be consumed.

MANSFELD'S (16) view is not essentially different from that of VERWORN. He believes that because the lipoids take up the narcotic, their power to absorb oxygen is decreased. Narcotized cells cannot take up sufficient oxygen for their needs and hence irritability is decreased by lack of oxygen. That narcotics do decrease the ability of olive oil to dissolve oxygen, has been asserted by HAMBURGER (8), although objection to his experiments has been made by WINTERSTEIN (31). In many cases it is certain that narcosis has nothing to do with absorption of oxygen. Thus WINTERSTEIN has observed that on anesthetizing the anaerobic worm *Ascaris* in absence of oxygen, it comes to rest very quickly under the influence of the anesthetic.

Experiments by numerous investigators have shown that narcosis and decrease of oxidation are not parallel. WARBURG (30) has observed that some narcotics decrease the oxidation of the erythrocytes of geese as much as 30-70 per cent. He has also observed, however, that narcotics do not always decrease the consumption of oxygen, for he has found that the segmentation of fertilized sea urchin eggs can be inhibited by phenylurethane without a perceptible decrease of the consumption of oxygen. LOEB and WASTENEYS (14) have obtained similar results with chloral hydrate, chloroform, and alcohol. NOTHMANN-ZUCKERKANDL (20) observed that the protoplasmic streaming of plant cells is quickly brought to a standstill by narcotics. Such cells have been shown to be rather insensitive to lack of oxygen, in that the streaming is stopped in the absence of oxygen only after several weeks.

BÜRKER (4) was of the opinion that because of the great solubility of narcotics in the lipid of the cells, there is a competition between the lipoids and other substances in the protoplasm for the active oxygen, such that the cells are more or less in a state of

asphyxiation. His hypothesis maintains that the absorption of oxygen is not reduced during narcosis, but that the oxygen is prevented from going to its usual point of attack. This would necessitate the assumption that oxygen is more readily absorbed by the lipoids than by the other substances of the cell, which is not the case. An objection to all theories which make narcosis conditional on lipid solubility is the fact that magnesium sulphate and carbon dioxide, which are not soluble in lipid, produce typical anesthesia.

HÖBER (10) has formulated the hypothesis that narcosis is due to inhibition of enzymatic processes, brought about by a decrease in dispersion. This hypothesis is favored by the investigations of BATELLI and STERN (2), who found that proteins, such as the nucleo-proteins, are influenced by narcotics in approximately the same relative and absolute concentrations as those at which the enzymes are affected. In this connection VERNON (28) found that most narcotics are harmless to oxidases up to a definite limiting concentration, beyond which injury occurs. HÖBER explains the retardation or inhibition of enzyme action as due to the fact that narcotics go into the surface between the enzymes and the medium in which they are dispersed, thereby displacing the substratum on which the enzymes act.

The investigations hitherto discussed are largely based on measurements of the absorption of oxygen. In many cases such measurements could be made more accurately than determinations of the amount of carbon dioxide produced. Since the writer has recently been able to develop a method for the measurement of minute amounts of carbon dioxide in solution, it seemed to him that a fresh investigation of the subject by means of this method was desirable.

Previous investigations on the effect of narcotics on the production of carbon dioxide have yielded somewhat contradictory results. APPLEMAN (1) found that vapor of ethyl bromide approximately doubles the respiration of potatoes. MAYER (18) found that 0.25 per cent prussic acid stops the respiration of higher plants entirely. SCHROEDER (24) observed a decrease in respiration when *Aspergillus* was treated with ether. He found that prussic acid

inhibited the production of CO_2 , while the absorption of oxygen continued. KOSINSKI (12) found that low concentrations of ether increased the respiration of *Aspergillus*, while higher concentrations decreased it. LAURÉN (13) and also IRVING (11) have noted that respiration increases during anesthesia produced by ether and chloroform. TASHIRO (26) found that anesthetics greatly reduce the output of CO_2 by dry seeds. BONNIER and MANGIN (3), as the result of experimental work upon the influence of measured quantities of ether upon flowering plants, concluded that the respiratory activity is unaffected by anesthetics. It has since been shown by EWART (6) that chloroform increases the respiratory activity in *Elodea*.

The effect of anesthetics upon the respiration of marine plants has received very little attention. HARDER (9) has made determinations of the respiration of marine algae, but in no case has he studied the effect of anesthetics. PANTANELLI (22) has observed that sea water, when half saturated with chloroform, reduces the excretion of CO_2 to about one-half of the normal. His experiments were very few, and no duplicate or control experiments were made. The methods employed by PANTANELLI and HARDER often required that plants be shut up air-tight, in flasks completely filled with sea water, and left in this condition for several hours, analyses being made at the beginning and end of these long periods.

In the experiments of the writer on the effect of anesthetics upon the production of CO_2 , the marine alga *Laminaria* was found to be well suited to the purpose. Fronds were cut up into pieces about 2 inches long. Each piece was rolled up loosely and inserted into a piece of Pyrex glass tubing. This was closed at one end, while a piece of paraffined rubber tubing was attached to the open end. Sea water, of approximately the same temperature as the material, was then added to the tubes and the rubber tube closed by a spring clamp. The tubes were then brought very gradually to the temperature (16°C.) of the constant temperature bath. The tubes were kept dark by inserting each tube in a black-enameled, collapsible tin tube submerged in the bath. Several tubes were used as controls in each experiment. These contained material in sea water without any addition. The reagents were

always used at the same temperature as that of the water bath. After the tubes containing the *Laminaria* in sea water had been at 16° C. from half an hour to an hour, the solution was poured out of each tube and replaced by fresh sea water at 16° C. This was repeated several times before beginning an experiment.

After a tube containing *Laminaria* in sea water had been clamped off so as to include a small bubble of air which could be used as a stirrer, and had been exposed to 16° C. for a definite period, it was removed from the bath. The solution was slightly stirred by inverting the tube a few times; the clamp was then opened and the solution rapidly poured into another empty tube to which the same number of drops of indicator (phenolsulphonephthalein) had been added as was added to the buffer solutions (7). In order to mix the solution with the indicator it was stirred as described, and was then compared with buffer solutions of a known PH value which contained the same amount of indicator.

The use of a small bubble of air as a stirring agent was found to be very convenient, and when compared with the use of paraffined glass globules as stirrers was found to introduce no error of any importance.

The buffer solutions (27) were made up by mixing M/15 Na_2HPO_4 and M/15 KH_2PO_4 in various proportions. They were of the same diameter as those containing the alga. A 0.01 per cent aqueous solution of phenolsulphonephthalein served as the indicator of the PH values. The indicator solution was used at the rate of 5 drops in 10 cc. of solution. A correction (30) of 0.30 for the salt error of the indicator in sea water was subtracted from the observed readings of the PH value of sea water. The use of a constant source of light (the "Daylight" lamp) permitted observations to be made in a uniform manner. The decrease in PH, which results from the production of CO_2 , served as a criterion of the amount of respiration.

In all the experiments, each tube was observed for a number of periods (always of the same length) for each piece of material, until it was evident that the rate of respiration had become practically constant. Several of the tubes were then used as controls, while to the others was added the sea water containing the

anesthetic. In every case tubes which contained only sea water showed that the apparatus was not responsible for any of the changes observed in the experiments in which tissue was used.

The solutions used in any one experiment were always of the same PH value, so that the results were comparable. Many of the anesthetics, especially when in small concentrations, do not appreciably affect the PH value of the sea water. A large number of carboys of sea water, obtained from Woods Hole at the same time, were filtered to remove organic matter in varying degrees, the unfiltered material settling to the bottom. When an anesthetic decreased the PH value only slightly, it was possible by a suitable selection of the sea water from the carboys to obtain a bottle of sea water of a PH value equal to that of the sea water containing the anesthetic.

Whenever large amounts of an anesthetic were used in the sea water, it was considered advisable to add concentrated sea water to bring the electrical conductivity of the solution up to that of sea water. This was done with each of the following solutions: sea water containing 16.1 and 24.2 per cent alcohol respectively; sea water containing 17.4 per cent acetone; and sea water containing 3.2 per cent formaldehyde. Many of the experiments were repeated at Woods Hole in the summer of 1917. In these experiments the solutions containing alcohol (all above 1 per cent) were made of the same electrical conductivity as sea water. The results were practically the same as when no concentrated sea water was added.

In the case of the higher concentration of formaldehyde, the free acid was first neutralized with sodium carbonate. This is allowable for the purposes of the present investigation, as its only effect would be to make the amount of CO_2 produced appear somewhat less than was actually the case. It should be understood that the percentages of liquid anesthetics in the following descriptions are percentages by volume. In an experiment in which there is considerable dilution, as when absolute alcohol is added to sea water to make a 10 per cent alcoholic solution, it might be conceivable that the dilution might be the cause of the increased rate of respiration. For that reason experiments were made with

pieces of material in normal sea water until constant values for the respiration were obtained; then sea water diluted with 10 per cent of tap water was used, and it was found that the dilution produced no appreciable effect.

In each experiment 6 cc. of solution was used. At the end of each period, after noting the new PH value, 6 cc. of fresh solution was poured into the tube, which was then treated as before. It was at first thought that in pouring out the solution from the tube containing the tissue sufficient might be held back by the tissue to affect the observed PH value. Actual determinations proved that this was not the case.

In view of the fact that there may be an increase of respiration as the result of injury (23), preliminary experiments were made to ascertain whether the cutting of the *Laminaria* had any appreciable effect upon the respiration. It was found that the change in the respiration due to cutting was negligible (the cutting was always reduced to a minimum).

In some of the experiments there was noted a very slight decrease in the production of CO_2 as time went on, although not enough to be of significance for the present investigation. This phenomenon has been observed by Miss MATTHAEI (17) in determinations of CO_2 in connection with experiments on photosynthesis. Such a decrease in respiration has been attributed by her to the gradual decrease of substances available for oxidation.

The results of the experiments are given both in tables and figures. In each experiment 6-12 (or more) closely agreeing determinations were obtained and the results of a typical case were taken. The plant was always placed for a definite time in sea water or sea water containing the reagent, and at the end of this time the PH value was determined. This interval is in every case shown in the tables as well as by the points on the curves. At the end of each interval the solution was renewed.

The alteration of the PH value is an index of the amount of CO_2 produced, since the greater the amount of CO_2 the greater the decrease in PH. Since this relation is approximately linear in this range of PH values, it is not necessary to translate the decrease in PH value into cc. of CO_2 produced, as the form of the respiration

curve would be practically the same whether we use as ordinates PH values or cc. of CO_2 produced. The curves are plotted in such a manner that the abscissae represent time (in minutes) and the ordinates represent the change in PH value corresponding to either the relative amount of respiration, the relative rate of respiration, or to both (as designated in the figures). The relative amount of respiration is obtained by dividing the total change in PH during exposure to the reagent by the total change in PH caused by the

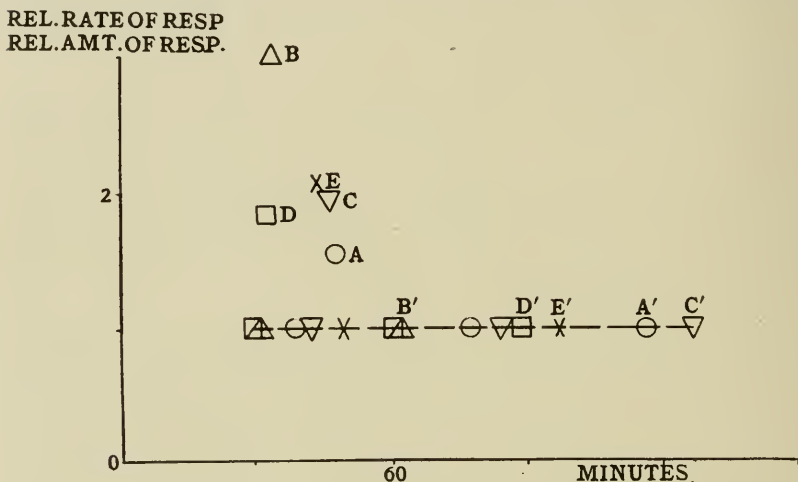


FIG. 1a.—Points showing relative rate and relative amount (identical in this case) of respiration of *Laminaria* produced by sea water containing A, 0.1 per cent chloral hydrate; B, 0.1 per cent novocain; C, 1 per cent ether; D, 0.1 per cent caffeine; E, ethyl-bromide; controls in sea water (broken lines); see tables I A to E; each control bears the same letter (with a prime) as the experimental curve.

same material under normal conditions during the same length of time. The relative rate is obtained by dividing the change in PH during one period by the change produced during a similar period by the same material under normal conditions. The broken lines in each case represent controls in sea water. The curve of each control bears the same symbol and letter as the experimental curve for sea water plus anesthetic, except that in the control the letters are primed.

Inspection of the results for 0.1 per cent chloral hydrate (fig. 1a [A]; table I A), 0.1 per cent novocain (fig. 1a [B]; table I B),

TABLE I A*

PERIOD OF 47.75 MIN. IN SEA WATER AND EQUAL PERIOD IN SEA WATER CONTAINING 0.1 PER CENT CHLORAL HYDRATE

Solution	Change in PH	Relative rate of respiration
Sea water.	$7.90 - 7.55 = 0.35$
Sea water containing 0.1 per cent chloral hydrate.	$7.90 - 7.35 = 0.55$	$0.55 \div 0.35 = 1.57$

* In the control, with 3 periods (38.75 min. each) in sea water, the change in PH in each period was $7.90 - 7.55 = 0.35$.

TABLE I B*

PERIOD OF 33.25 MIN. IN SEA WATER FOLLOWED BY EQUAL PERIOD IN SEA WATER CONTAINING 0.1 PER CENT NOVOCAIN

Solution	Change in PH	Relative rate of respiration
Sea water.	$7.65 - 7.40 = 0.25$
Sea water containing 0.1 per cent novocain.	$7.65 - 6.90 = 0.75$	$0.75 \div 0.25 = 3$

* In the control, with 2 periods (30.75 min. each) in sea water, the change in PH in each period was $7.65 - 7.40 = 0.25$.

TABLE I C*

PERIOD OF 45.25 MIN. IN SEA WATER AND EQUAL PERIOD IN SEA WATER CONTAINING 1 PER CENT (BY VOLUME) OF ETHER

Solution	Change in PH	Relative rate of respiration
Sea water.	$7.90 - 7.57 = 0.33$
Sea water containing 1 per cent ether	$7.90 - 7.25 = 0.65$	$0.65 \div 0.33 = 1.97$

* In the control, with 3 periods (42 min. each) in sea water, the change in PH in each period was $7.90 - 7.60 = 0.30$.

TABLE I D*

TWO PERIODS (32.5 MIN. EACH) IN SEA WATER AND SAME LENGTH OF TIME IN SEA WATER CONTAINING 0.1 PER CENT CAFFEINE

Solution	Change in PH	Relative rate of respiration
Sea water.	$7.65 - 7.43 = 0.22$
" "	$7.65 - 7.43 = 0.22$
Sea water containing 0.1 per cent caffeine.	$7.65 - 7.25 = 0.40$	$0.40 \div 0.22 = 1.8$

* In the control, with 3 periods (29.5 min. each) in sea water, the change in PH in each period was $7.65 - 7.50 = 0.15$.

TABLE I E*

PERIOD (43.25 MIN.) IN SEA WATER FOLLOWED BY EQUAL PERIOD IN SEA WATER
APPROXIMATELY SATURATED WITH ETHYL BROMIDE

Solution	Change in PH	Relative rate of respiration
Sea water.....	$7.90 - 7.35 = 0.55$
Sea water saturated with ethyl bromide.....	$7.90 - 6.75 = 1.15$	$1.15 \div 0.55 = 2.1$

* In the control, with 2 periods (49.25 min. each) in sea water, the change in PH in each period was $7.90 - 7.30 = 0.60$.

TABLE I F

THREE PERIODS (22 MIN. EACH) IN SEA WATER AND 7 EQUAL PERIODS IN SEA
WATER APPROXIMATELY SATURATED WITH ETHYL BROMIDE

Solution	Change in PH	Relative rate of respiration
Sea water.....	$8.1 - 7.8 = 0.30$
" ".....	$8.1 - 7.8 = 0.30$
Sea water containing ethyl bromide	$7.32 - 6.16^* = 1.16 +$	$1.16 \div 0.30 = 3.8$
" " " " "	$7.32 - 6.16^* = 1.16 +$	$1.16 \div 0.30 = 3.8$
" " " " "	$7.32 - 6.69 = 0.63$	$0.63 \div 0.30 = 2.1$
" " " " "	$7.32 - 7.07 = 0.25$	$0.25 \div 0.30 = 0.8$
" " " " "	$7.32 - 7.21 = 0.11$	$0.11 \div 0.30 = 0.4$
" " " " "	$7.32 - 7.32 = 0.0$	$0.0 \div 0.30 = 0.0$

* Approximate (at this point the indicator is not very sensitive to slight changes in acidity).

TABLE I F CONTROL

SEVEN PERIODS (27.5 MIN.) IN SEA WATER

Period	Change in PH	Relative rate of respiration
1.....	$8.1 - 7.6 = 0.5$	$0.5 \div 0.5 = 1.00$
2.....	$8.1 - 7.6 = 0.5$	$0.5 \div 0.5 = 1.00$
3.....	$8.1 - 7.6 = 0.5$	$0.5 \div 0.5 = 1.00$
4.....	$8.1 - 7.6 = 0.5$	$0.5 \div 0.5 = 1.00$
5.....	$8.1 - 7.65 = 0.45$	$0.45 \div 0.5 = 0.90$
6.....	$8.1 - 7.65 = 0.45$	$0.45 \div 0.5 = 0.90$
7.....	$8.1 - 7.65 = 0.45$	$0.45 \div 0.5 = 0.90$

1 per cent ether (fig. 1a [C]; table I C), 0.1 per cent caffeine (fig. 1a [D]; table I D), and for sea water approximately saturated with ethyl bromide (fig. 1a [E]; table I E; fig. 1b [A and B]; table I F) shows that these anesthetics increase both the relative amount and relative rate of respiration.

In fig. 1*b* (A) it will be seen that in sea water containing ethyl bromide the relative rate of respiration is greatly increased during the first 2 periods and then drops to the normal rate in about 90 min. After 132 min. no excretion of CO₂ could be detected. When we plot the curve B (fig. 1*b*), in which the ordinates represent the relative amount of respiration, we find that the curve is far above the base line even when the respiration cannot be detected.

REL. RATE OF RESP.
REL. AMT. OF RESP.

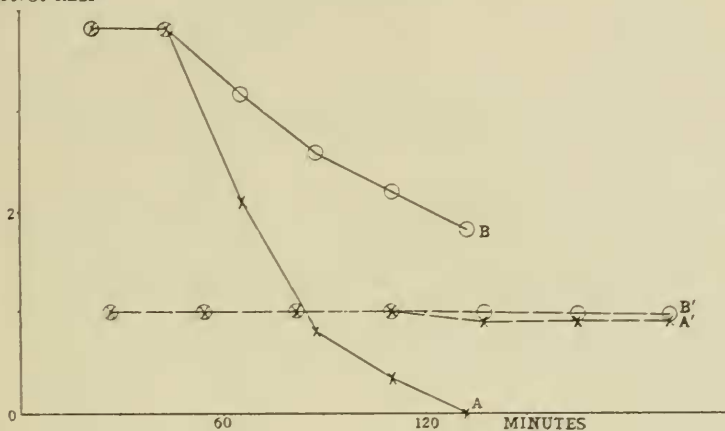


FIG. 1*b*.—Curves showing effect of sea water approximately saturated with ethyl bromide upon A, relative rate of respiration; B, relative amount of respiration of *Laminaria* (unbroken lines); controls in sea water (broken lines); see table I F; each control bears the same letter (with a prime) as the experimental curve.

The fact that ethyl bromide appears to have a marked accelerating effect upon the respiration confirms the results of APPLEMAN (1), who observed that when potato tubers are exposed to ethyl bromide vapor the respiration is greatly increased. Fig. 1*b* makes it evident that there is no initial decrease in the respiration.

The ethyl bromide referred to in table I F was acid, and since it was not neutralized by adding sodium carbonate it caused the PH of the sea water plus anesthetic to be lower than that of the sea water alone. In experiments with all other substances both sea water and sea water plus anesthetic were of the same PH value at the beginning of the experiment.

The respiration of *Laminaria* was followed for several hours, when the tissue was placed in solutions of sea water containing 3.2 per cent (fig. 2a [A], fig. 2b [A]; table II A) and 0.8 per cent

REL. AMT. OF RESP.

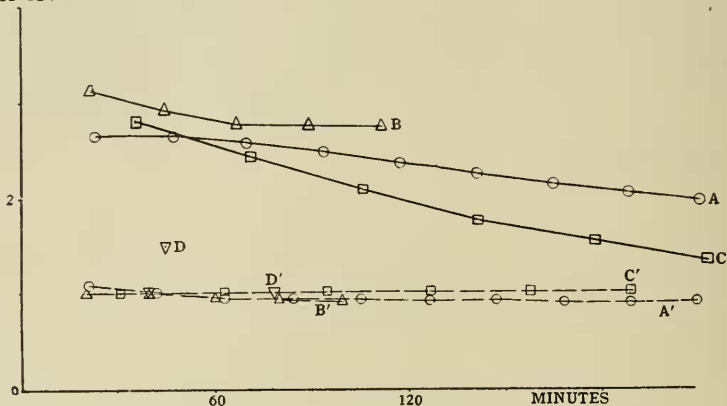


FIG. 2a.—Curves (unbroken lines) showing the effect upon the relative amount of respiration of *Laminaria* of sea water containing A, 3.2 per cent formaldehyde; B, 0.8 per cent formaldehyde; C, 0.3 per cent chloroform; D, 0.05 per cent chloroform; controls in sea water (broken lines); see tables II A to II D; each control bears the same letter (with a prime) as the experimental curve.

REL. RATE OF RESP.

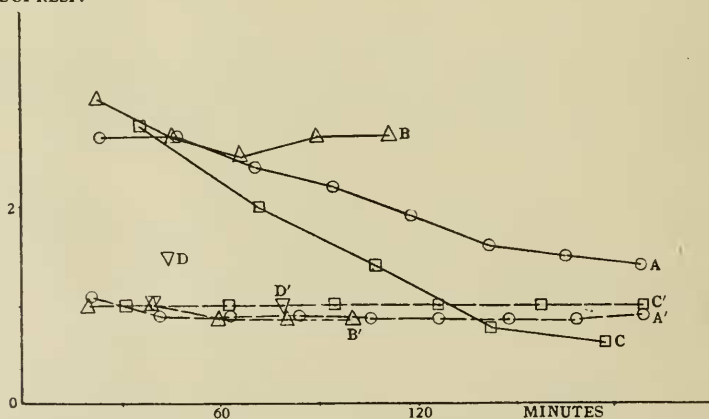


FIG. 2b.—Curves (unbroken lines) showing effect upon relative rate of respiration of *Laminaria* of sea water containing A, 3.2 per cent formaldehyde; B, 0.8 per cent formaldehyde; C, 0.3 per cent chloroform; D, 0.05 per cent chloroform; controls in sea water (broken lines); each control bears the same letter (with a prime) as the experimental curve.

TABLE II A

TWO PERIODS (23.5 MIN. EACH) IN SEA WATER AND 13 EQUAL PERIODS IN SEA WATER CONTAINING 3.2 PER CENT FORMALDEHYDE (8 PER CENT BY VOLUME OF 40 PER CENT FORMALDEHYDE)

Solution	Change in PH	Relative rate of respiration
Sea water.....	$8.37-7.82=0.55$
" ".....	$8.37-7.82=0.55$
Sea water containing 3.2 per cent formaldehyde.....	$8.37-6.90=1.47$	$1.47 \div 0.55 = 2.7$
Sea water containing 3.2 per cent formaldehyde.....	$8.37-6.90=1.47$	$1.47 \div 0.55 = 2.7$
Sea water containing 3.2 per cent formaldehyde.....	$8.37-7.05=1.32$	$1.32 \div 0.55 = 2.4$
Sea water containing 3.2 per cent formaldehyde.....	$8.37-7.15=1.22$	$1.22 \div 0.55 = 2.2$
Sea water containing 3.2 per cent formaldehyde.....	$8.37-7.33=1.04$	$1.04 \div 0.55 = 1.9$
Sea water containing 3.2 per cent formaldehyde.....	$8.37-7.50=0.87$	$0.87 \div 0.55 = 1.6$
Sea water containing 3.2 per cent formaldehyde.....	$8.37-7.55=0.82$	$0.82 \div 0.55 = 1.5$
Sea water containing 3.2 per cent formaldehyde.....	$8.37-7.58=0.79$	$0.79 \div 0.55 = 1.4$
Sea water containing 3.2 per cent formaldehyde.....	$8.37-7.60=0.77$	$0.77 \div 0.55 = 1.4$
Sea water containing 3.2 per cent formaldehyde.....	$8.37-7.65=0.72$	$0.72 \div 0.55 = 1.3$
Sea water containing 3.2 per cent formaldehyde.....	$8.37-7.70=0.67$	$0.67 \div 0.55 = 1.2$
Sea water containing 3.2 per cent formaldehyde.....	$8.37-7.80=0.57$	$0.57 \div 0.55 = 1.0$
Sea water containing 3.2 per cent formaldehyde.....	$8.37-7.85=0.52$	$0.52 \div 0.55 = 0.95$

TABLE II A CONTROL

FIFTEEN PERIODS (21 MIN.) IN SEA WATER

Period	Change in PH	Relative rate of respiration
1.....	$8.37-7.80=0.57$	$0.57 \div 0.52 = 1.10$
2.....	$8.37-7.90=0.47$	$0.47 \div 0.52 = 0.90$
3.....	$8.37-7.90=0.47$	$0.47 \div 0.52 = 0.90$
4.....	$8.37-7.90=0.47$	$0.47 \div 0.52 = 0.90$
5.....	$8.37-7.92=0.45$	$0.45 \div 0.52 = 0.87$
6.....	$8.37-7.92=0.45$	$0.45 \div 0.52 = 0.87$
7.....	$8.37-7.92=0.45$	$0.45 \div 0.52 = 0.87$
8.....	$8.37-7.92=0.45$	$0.45 \div 0.52 = 0.87$
9.....	$8.37-7.90=0.47$	$0.47 \div 0.52 = 0.90$
10.....	$8.37-7.90=0.47$	$0.47 \div 0.52 = 0.90$
11.....	$8.37-7.90=0.47$	$0.47 \div 0.52 = 0.90$
12.....	$8.37-7.90=0.47$	$0.47 \div 0.52 = 0.90$
13.....	$8.37-7.90=0.47$	$0.47 \div 0.52 = 0.90$
14.....	$8.37-7.90=0.47$	$0.47 \div 0.52 = 0.90$
15.....	$8.37-7.90=0.47$	$0.47 \div 0.52 = 0.90$

TABLE II B

TWO PERIODS (22.25 MIN. EACH) IN SEA WATER FOLLOWED BY 5 EQUAL PERIODS IN SEA WATER CONTAINING 0.8 PER CENT FORMALDEHYDE (2 PER CENT BY VOLUME OF 40 PER CENT FORMALDEHYDE)

Solution	Change in PH	Relative rate of respiration
Sea water.....	$7.90-7.55=0.35$
" ".....	$7.90-7.55=0.35$
Sea water containing 0.8 per cent formaldehyde.....	$7.90-6.81=1.09$	$1.09 \div 0.35=3.1$
Sea water containing 0.8 per cent formaldehyde.....	$7.90-6.96=0.94$	$0.94 \div 0.35=2.7$
Sea water containing 0.8 per cent formaldehyde.....	$7.90-7.03=0.87$	$0.87 \div 0.35=2.5$
Sea water containing 0.8 per cent formaldehyde.....	$7.90-6.95=0.95$	$0.95 \div 0.35=2.7$
Sea water containing 0.8 per cent formaldehyde.....	$7.90-6.95=0.95$	$0.95 \div 0.35=2.7$

TABLE II B CONTROL

FIVE PERIODS (20 MIN.) IN SEA WATER

Period	Change in PH	Relative rate of respiration
1.....	$7.90-7.50=0.40$	$0.40 \div 0.40=1.00$
2.....	$7.90-7.50=0.40$	$0.40 \div 0.40=1.00$
3.....	$7.90-7.55=0.35$	$0.35 \div 0.40=0.87$
4.....	$7.90-7.55=0.35$	$0.35 \div 0.40=0.87$
5.....	$7.90-7.55=0.35$	$0.35 \div 0.40=0.87$

TABLE II C*

TWO PERIODS (35.5 MIN. EACH) IN SEA WATER AND DURING 6 EQUAL PERIODS IN SEA WATER CONTAINING 0.3 PER CENT (BY VOLUME) OF CHLOROFORM

Solution	Change in PH	Relative rate of respiration
Sea water.....	$8.03-7.43=0.60$
" ".....	$8.03-7.40=0.63$
Sea water containing 0.3 per cent chloroform.....	$8.03-6.30^\dagger=1.73$	$1.73 \div 0.615=2.8$
Sea water containing 0.3 per cent chloroform.....	$8.03-6.78=1.25$	$1.25 \div 0.615=2.0$
Sea water containing 0.3 per cent chloroform.....	$8.03-7.15=0.88$	$0.88 \div 0.615=1.4$
Sea water containing 0.3 per cent chloroform.....	$8.03-7.55=0.48$	$0.48 \div 0.615=0.78$
Sea water containing 0.3 per cent chloroform.....	$8.03-7.65=0.38$	$0.38 \div 0.615=0.62$
Sea water containing 0.3 per cent chloroform.....	$8.03-7.85=0.18$	$0.18 \div 0.615=0.29$

* In the control, with 8 periods (31.5 min. each) in sea water, the change in PH in each period was $8.03-7.80=0.23$.

† Approximate (at this point the indicator is not very sensitive to changes in PH).

TABLE II D*

ONE PERIOD (44.5 MIN.) IN SEA WATER FOLLOWED BY EQUAL PERIOD IN SEA WATER CONTAINING 0.5 PER CENT (BY VOLUME) OF CHLOROFORM

Solution	Change in PH	Relative rate of respiration
Sea water.....	$8.10 - 7.35 = 0.75$
Sea water containing 0.05 per cent chloroform.....	$8.10 - 6.97 = 1.13$	$1.13 \div 0.75 = 1.5$

* In the control, with 2 periods (39.25 min. each) in sea water, the change in PH in each period was $8.10 - 7.35 = 0.75$.

(fig. 2a [B], fig. 2b [B]; table II B) of formaldehyde respectively. In both figs. 2a and 2b (A) and (B), there is a marked increase in the respiration during the first period. The curves for the weaker concentration tend to become approximately horizontal in the later periods. The curves for the stronger concentration of formaldehyde present a somewhat different case. Here the respiration reaches its maximum during the first period and maintains this rate during the second period. Following this, the respiratory rate steadily becomes smaller.

The curves for 0.3 per cent (fig. 2a [C] and fig. 2b [C]; table II C) and 0.5 per cent (fig. 2a [D] and fig. 2b [D]; table II D) chloroform respectively, each show that respiration is increased during the first period. The curves for the 0.3 per cent chloroform indicate that the rate steadily becomes smaller, until at the end of about 2.25 hours the respiratory rate falls below what it was normally (when in sea water).

The observation has often been made (11) that in human beings and in mammals during prolonged anesthesia there are typical products of incomplete oxidation such as fatty acids, lactic acid, and above all acetone (in not inconsiderable quantities) eliminated, as the case may be, into the urine or into the respired air. It seemed of interest in this connection to study the effect of acetone upon the respiration of *Laminaria*. It will be seen from the curves that when sea water contains 0.1 per cent (fig. 3 [A]; table III A) or 0.51 per cent (fig. 3 [B]; table III B) of acetone respectively, the respiration is practically unaffected. When, however, the sea water contains 17.4 per cent of acetone (fig. 3 [C] and [D]; table III C), a peculiar condition results. During the first period the

respiratory rate (curve D) is greatly increased and reaches its maximum during the second period. This is followed by a rapid decrease in the rate during the third and fourth periods, although the rate is still above the normal. After the fourth period a more

REL. RATE OF RESP.
REL. AMT. OF RESP.

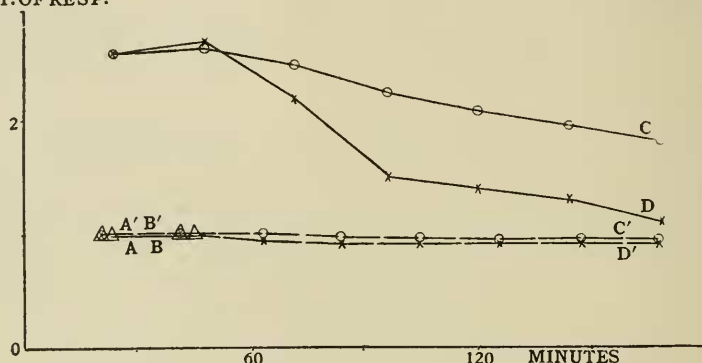


FIG. 3.—Curves (unbroken lines) showing effect of sea water containing A, 0.1 per cent acetone; B, 0.51 per cent acetone upon relative rate and relative amount of respiration of *Laminaria* (identical for these substances); C, effect of sea water containing 17.4 per cent acetone (unbroken line) upon relative amount of respiration; D, effect of sea water containing 17.4 per cent acetone (unbroken line) upon relative rate of respiration; controls in sea water (broken lines); see tables III A to III C; each control bears the same letter (with a prime) as the experimental curve.

gradual decline begins, so that even at the end of the experiment covering 2 hours and 48 min. the respiratory rate is still slightly above the normal.

TABLE III A AND B

TWO PERIODS (23 MIN. EACH) IN SEA WATER FOLLOWED BY 2 EQUAL PERIODS IN SEA WATER CONTAINING 0.1 PER CENT ACETONE, FOLLOWED BY 2 SIMILAR PERIODS IN SEA WATER CONTAINING 0.51 PER CENT ACETONE

Solution	Change in PH	Relative rate of respiration
Sea water.....	8.37-7.64=0.73
" ".....	8.37-7.64=0.73
Sea water containing 0.1 per cent acetone.....	8.37-7.65=0.72	$0.72 \div 0.73 = 0.99$
Sea water containing 0.1 per cent acetone.....	8.37-7.65=0.72	$0.72 \div 0.73 = 0.99$
Sea water containing 0.51 per cent acetone.....	8.37-7.65=0.72	$0.72 \div 0.73 = 0.99$
Sea water containing 0.51 per cent acetone.....	8.37-7.63=0.74	$0.74 \div 0.73 = 1.01$

TABLE III A AND B CONTROL
SIX PERIODS (20.25 MIN. EACH) IN SEA WATER

Period	Change in PH	Relative rate of respiration
1.....	$8.37-7.60=0.77$	$0.77 \div 0.77 = 1.00$
2.....	$8.37-7.60=0.77$	$0.77 \div 0.77 = 1.00$
3.....	$8.37-7.61=0.76$	$0.76 \div 0.77 = 0.99$
4.....	$8.37-7.60=0.77$	$0.77 \div 0.77 = 1.00$
5.....	$8.37-7.60=0.77$	$0.77 \div 0.77 = 1.00$
6.....	$8.37-7.60=0.77$	$0.77 \div 0.77 = 1.00$

TABLE III C

TWO PERIODS (24 MIN. EACH) IN SEA WATER AND 7 EQUAL PERIODS IN SEA WATER
CONTAINING 17.4 PER CENT (BY VOLUME) OF ACETONE

Solution	Change in PH	Relative rate of respiration
Sea water.....	$8.37-7.76=0.61$
" ".....	$8.37-7.78=0.59$
Sea water containing 17.4 per cent acetone.....	$8.37-6.80=1.57$	$1.57 \div 0.60 = 2.6$
Sea water containing 17.4 per cent acetone.....	$8.37-6.75=1.62$	$1.62 \div 0.60 = 2.7$
Sea water containing 17.4 per cent acetone.....	$8.37-7.06=1.31$	$1.31 \div 0.60 = 2.2$
Sea water containing 17.4 per cent acetone.....	$8.37-7.50=0.87$	$0.87 \div 0.60 = 1.5$
Sea water containing 17.4 per cent acetone.....	$8.37-7.54=0.83$	$0.83 \div 0.60 = 1.4$
Sea water containing 17.4 per cent acetone.....	$8.37-7.62=0.75$	$0.75 \div 0.60 = 1.3$
Sea water containing 17.4 per cent acetone.....	$8.37-7.74=0.63$	$0.63 \div 0.60 = 1.1$

TABLE III C CONTROL
NINE PERIODS (21 MIN. EACH) IN SEA WATER

Period	Change in PH	Relative rate of respiration
1.....	$8.37-7.67=0.70$	$0.70 \div 0.70 = 1.00$
2.....	$8.37-7.67=0.70$	$0.70 \div 0.70 = 1.00$
3.....	$8.37-7.70=0.67$	$0.67 \div 0.70 = 0.95$
4.....	$8.37-7.73=0.64$	$0.64 \div 0.70 = 0.91$
5.....	$8.37-7.73=0.64$	$0.64 \div 0.70 = 0.91$
6.....	$8.37-7.73=0.64$	$0.64 \div 0.70 = 0.91$
7.....	$8.37-7.72=0.65$	$0.65 \div 0.70 = 0.93$
8.....	$8.37-7.73=0.64$	$0.64 \div 0.70 = 0.91$
9.....	$8.37-7.72=0.65$	$0.65 \div 0.70 = 0.93$

In view of the fact that alcohol is considered to be formed during respiration, it was deemed important to study the effect of varying concentrations of alcohol upon the rate of respiration. When sea water contains 1 per cent of Squibb's absolute alcohol (figs. 4a [F] and 4b [F]; table IV F), the respiratory rate remains normal for 3 periods, after which there is a gradual decline to below

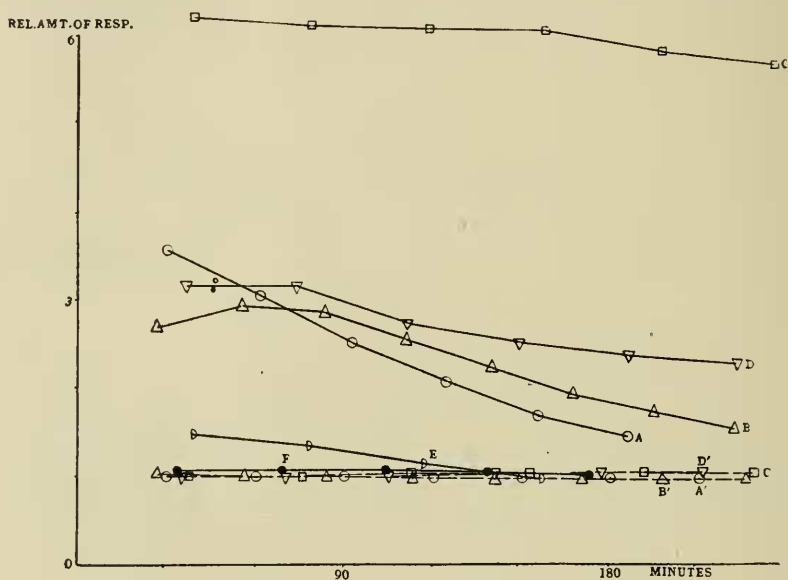


FIG. 4a.—Curves (unbroken lines) showing effect upon relative amount of respiration of *Laminaria* of sea water containing A, 24.2 per cent ethyl alcohol; B, 16.1 per cent ethyl alcohol; C, 10 per cent ethyl alcohol; D, 5 per cent ethyl alcohol; E, 2 per cent ethyl alcohol; F, 1 per cent ethyl alcohol; controls in sea water (broken lines); each control bears same letter (with prime) as the experimental curve, except that D' serves as control for curves D, E, and F; see tables IV A to IV F.

the normal rate, whereas the relative amount of respiration remains nearly constant. The curves for sea water containing 2 per cent alcohol (figs. 4a [E] and 4b [E]; table IV E) show a slight increase in the relative rate during the first period, followed by a smaller increase for the second period, after which there is a decline below the normal. As would be expected, 5 per cent alcohol (figs. 4a [D] and 4b [D]; table IV D) gives a much greater increase in the rela-

tive respiratory rate and amount than does 2 per cent alcohol (curve E). Unlike the 2 per cent alcohol, the maximum increase is maintained during the second period. The rate becomes less during the third period, after which it becomes quite constant. The curves for 10 per cent alcohol (figs. 4a [C] and 4b [C]; table IV C) reach their maximum during the first period. Approximately the

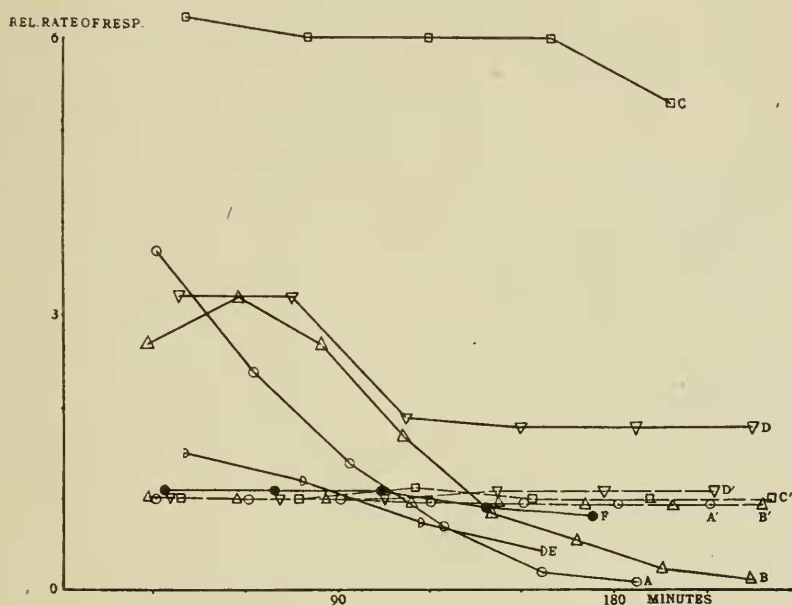


FIG. 4b.—Curves (unbroken lines) showing effect upon relative rate of respiration of *Laminaria* of sea water containing A, 24.2 per cent ethyl alcohol; B, 16.1 per cent ethyl alcohol; C, 10 per cent ethyl alcohol; D, 5 per cent ethyl alcohol; E, 2 per cent ethyl alcohol; F, 1 per cent ethyl alcohol; controls in sea water (broken lines); each control bears same letter (with prime) as the experimental curve, except that D' serves as control for curves D, E, and F; see tables IV A to IV F.

same rate of respiration is maintained for 3 periods, after which the increased rate rapidly becomes smaller. The relative amount of respiration remains approximately constant for 4 periods and then falls off very gradually. With 16.1 per cent alcohol (figs. 4a [B] and 4b [B]; table IV B) the maximum rate is not reached until the second period, after which the decline is more rapid than that for any of the lower concentrations. It will be seen that at the end of

224 min. the relative amount is about 1.5, while the relative rate falls below the normal after about 130 min. The maximum increased rate, when 24.2 per cent alcohol is used (figs. 4a [A] and 4b [A]; table IV A), is reached during the first period and then becomes smaller very much more rapidly than at any of the lower

TABLE IV A

TWO PERIODS (31.25 MIN. EACH) IN SEA WATER AND 6 EQUAL PERIODS IN SEA WATER CONTAINING 24.2 PER CENT (BY VOLUME) OF ETHYL ALCOHOL

Solution	Change in PH	Relative rate of respiration
Sea water.....	7.90-7.43 = 0.47
" ".....	7.90-7.43 = 0.47
Sea water containing 24.2 per cent ethyl alcohol.....	7.90-6.16* = 1.74	1.74 ÷ 0.47 = 3.7
Sea water containing 24.2 per cent ethyl alcohol.....	7.90-6.75 = 1.15	1.15 ÷ 0.47 = 2.4
Sea water containing 24.2 per cent ethyl alcohol.....	7.90-7.25 = 0.65	0.65 ÷ 0.47 = 1.4
Sea water containing 24.2 per cent ethyl alcohol.....	7.90-7.55 = 0.35	0.35 ÷ 0.47 = 0.7
Sea water containing 24.2 per cent ethyl alcohol.....	7.90-7.80 = 0.10	0.10 ÷ 0.47 = 0.2
Sea water containing 24.2 per cent ethyl alcohol.....	7.90-7.84 = 0.06	0.06 ÷ 0.47 = 0.1

* Approximate (at this point the indicator is not very sensitive to changes in PH).

TABLE IV A CONTROL

EIGHT PERIODS (30.25 MIN. EACH) IN SEA WATER

Period	Change in PH	Relative rate of respiration
1.....	7.90-7.53 = 0.37	0.37 ÷ 0.37 = 1.0
2.....	7.90-7.53 = 0.37	0.37 ÷ 0.37 = 1.0
3.....	7.90-7.53 = 0.37	0.37 ÷ 0.37 = 1.0
4.....	7.90-7.54 = 0.36	0.36 ÷ 0.37 = 0.97
5.....	7.90-7.54 = 0.36	0.36 ÷ 0.37 = 0.97
6.....	7.90-7.55 = 0.35	0.35 ÷ 0.37 = 0.95
7.....	7.90-7.55 = 0.35	0.35 ÷ 0.37 = 0.95
8.....	7.90-7.58 = 0.32	0.32 ÷ 0.37 = 0.87

concentrations, falling below the normal rate at about 110 min. The curve for relative amount of respiration at the end of 3 hours is about 1.4, even though respiration has nearly ceased.

These curves, showing the effect of varying concentrations of alcohol upon the rate of respiration, indicate that for concentrations above 1 per cent there is a marked increase in the respiratory

activity. Furthermore, there is a maximum increase which is usually reached in the first, but sometimes not until the second period. The relative amount and relative rate of respiration increase with increasing concentrations of ethyl alcohol up to 10

TABLE IV B

TWO PERIODS (28 MIN. EACH) IN SEA WATER FOLLOWED BY 8 EQUAL PERIODS IN SEA WATER CONTAINING 16.1 PER CENT ETHYL ALCOHOL

Solution	Change in PH	Relative rate of respiration
Sea water.....	$7.95-7.50=0.45$
" ".....	$7.95-7.52=0.43$
Sea water containing 16.1 per cent ethyl alcohol.....	$7.95-6.76=1.19$	$1.19 \div 0.44 = 2.7$
Sea water containing 16.1 per cent ethyl alcohol.....	$7.95-6.55=1.40$	$1.4 \div 0.44 = 3.2$
Sea water containing 16.1 per cent ethyl alcohol.....	$7.95-6.75=1.20$	$1.2 \div 0.44 = 2.7$
Sea water containing 16.1 per cent ethyl alcohol.....	$7.95-7.20=0.75$	$0.75 \div 0.44 = 1.7$
Sea water containing 16.1 per cent ethyl alcohol.....	$7.95-7.57=0.38$	$0.38 \div 0.44 = 0.86$
Sea water containing 16.1 per cent ethyl alcohol.....	$7.95-7.70=0.25$	$0.25 \div 0.44 = 0.57$
Sea water containing 16.1 per cent ethyl alcohol.....	$7.95-7.85=0.10$	$0.10 \div 0.44 = 0.23$
Sea water containing 16.1 per cent ethyl alcohol.....	$7.95-7.90=0.05$	$0.05 \div 0.44 = 0.11$

TABLE IV B CONTROL

NINE PERIODS (28.5 MIN. EACH) IN SEA WATER

Period	Change in PH	Relative rate of respiration
1.....	$7.95-7.55=0.40$	$0.40 \div 0.395 = 1.01$
2.....	$7.95-7.50=0.39$	$0.39 \div 0.395 = 0.99$
3.....	$7.95-7.56=0.39$	$0.39 \div 0.395 = 0.99$
4.....	$7.95-7.57=0.38$	$0.38 \div 0.395 = 0.96$
5.....	$7.95-7.58=0.37$	$0.37 \div 0.395 = 0.94$
6.....	$7.95-7.58=0.37$	$0.37 \div 0.395 = 0.94$
7.....	$7.95-7.58=0.37$	$0.37 \div 0.395 = 0.94$
8.....	$7.95-7.58=0.37$	$0.37 \div 0.395 = 0.94$
9.....	$7.95-7.58=0.37$	$0.37 \div 0.395 = 0.94$

per cent. At concentrations from 2 to 10 per cent the relative rate of respiration remained far above the normal during the entire experiment. When larger concentrations such as 16.1 or 24 per cent are used, however, the decline, once the maximum rate has been reached, becomes more rapid with increasing concentrations

of alcohol. The curves of the relative rate of respiration for such higher concentrations of alcohol fall quite rapidly below the normal, whereas the curves for the relative amount of respiration for the same experiment may remain far above unity.

TABLE IV C

TWO PERIODS (39.75 MIN. EACH) IN SEA WATER FOLLOWED BY 6 EQUAL PERIODS IN SEA WATER CONTAINING 10 PER CENT ETHYL ALCOHOL

Solution	Change in PH	Relative rate of respiration
Sea water.....	$7.70-7.55=0.15$
" ".....	$7.70-7.55=0.15$
Sea water containing 10 per cent ethyl alcohol.....	$7.70-6.77=0.93$	$0.93 \div 0.15 = 6.2$
Sea water containing 10 per cent ethyl alcohol.....	$7.70-6.80=0.90$	$0.90 \div 0.15 = 6.0$
Sea water containing 10 per cent ethyl alcohol.....	$7.70-6.80=0.90$	$0.90 \div 0.15 = 6.0$
Sea water containing 10 per cent ethyl alcohol.....	$7.70-6.80=0.90$	$0.90 \div 0.15 = 6.0$
Sea water containing 10 per cent ethyl alcohol.....	$7.70-6.90=0.80$	$0.80 \div 0.15 = 5.3$
Sea water containing 10 per cent ethyl alcohol.....	$7.70-7.05=0.65$	$0.65 \div 0.15 = 4.3$

TABLE IV C CONTROL

EIGHT PERIODS (38.5 MIN. EACH) IN SEA WATER

Period	Change in PH	Relative rate of respiration
1.....	$7.70-7.55=0.15$	$0.15 \div 0.15 = 1.00$
2.....	$7.70-7.55=0.15$	$0.15 \div 0.15 = 1.00$
3.....	$7.70-7.53=0.17$	$0.17 \div 0.15 = 1.13$
4.....	$7.70-7.55=0.15$	$0.15 \div 0.15 = 1.00$
5.....	$7.70-7.55=0.15$	$0.15 \div 0.15 = 1.00$
6.....	$7.70-7.55=0.15$	$0.15 \div 0.15 = 1.00$
7.....	$7.70-7.55=0.15$	$0.15 \div 0.15 = 1.00$
8.....	$7.70-7.55=0.15$	$0.15 \div 0.15 = 1.00$

It is important to know whether or not the decrease in PH value is due to the excretion of CO_2 or to other acids, such as organic acids which are products of incomplete oxidation. To determine this pure hydrogen was bubbled through the solution (which has been made more acid by *Laminaria*) until the excess of CO_2 was expelled. The solution was then allowed to come into equilibrium with the CO_2 of the air. It was found that in all the experiments

(with certain exceptions to be mentioned) the color of the indicator was reversible by this means. This showed conclusively that the increased acidity was actually due to CO_2 . In the case of sea water containing 0.3 per cent chloroform, the color of the indicator

TABLE IV D

TWO PERIODS (37.5 MIN. EACH) IN SEA WATER FOLLOWED BY 6 EQUAL PERIODS IN SEA WATER CONTAINING 5 PER CENT ETHYL ALCOHOL

Solution	Change in PH	Relative rate of respiration
Sea water.....	$7.65 - 7.43 = 0.22$
" ".....	$7.65 - 7.43 = 0.22$
Sea water containing 5 per cent ethyl alcohol.....	$7.65 - 6.95 = 0.70$	$0.70 \div 0.22 = 3.2$
Sea water containing 5 per cent ethyl alcohol.....	$7.65 - 6.95 = 0.70$	$0.70 \div 0.22 = 3.2$
Sea water containing 5 per cent ethyl alcohol.....	$7.65 - 7.22 = 0.43$	$0.43 \div 0.22 = 1.9$
Sea water containing 5 per cent ethyl alcohol.....	$7.65 - 7.25 = 0.40$	$0.40 \div 0.22 = 1.8$
Sea water containing 5 per cent ethyl alcohol.....	$7.65 - 7.25 = 0.40$	$0.40 \div 0.22 = 1.8$
Sea water containing 5 per cent ethyl alcohol.....	$7.65 - 7.25 = 0.40$	$0.40 \div 0.22 = 1.8$

TABLE IV E

TWO PERIODS (39.25 MIN. EACH) IN SEA WATER FOLLOWED BY 4 EQUAL PERIODS IN SEA WATER CONTAINING 2 PER CENT ETHYL ALCOHOL

Solution	Change in PH	Relative rate of respiration
Sea water.....	$7.65 - 7.35 = 0.30$
" ".....	$7.65 - 7.35 = 0.30$
Sea water containing 2 per cent ethyl alcohol.....	$7.65 - 7.20 = 0.45$	$0.45 \div 0.30 = 1.5$
Sea water containing 2 per cent ethyl alcohol.....	$7.65 - 7.28 = 0.37$	$0.37 \div 0.30 = 1.2$
Sea water containing 2 per cent ethyl alcohol.....	$7.65 - 7.43 = 0.22$	$0.22 \div 0.30 = 0.73$
Sea water containing 2 per cent ethyl alcohol.....	$7.65 - 7.52 = 0.13$	$0.13 \div 0.30 = 0.43$

was not reversible at the end of the first period, although after any of the succeeding periods the color was fully reversible. The color of the indicator in sea water containing 17.4 per cent acetone (made up to the conductivity of sea water) was not reversible at the end of the first period of exposure. At the end of the second period of

exposure the color was almost entirely reversible, and after the third period (and succeeding periods) the color was completely reversible. When sea water containing 24.2 per cent ethyl alcohol (made up to conductivity of sea water) was used, the color of the indicator after the first period of exposure was not reversible. The

TABLE IV F

TWO PERIODS (34.75 MIN. EACH) IN SEA WATER FOLLOWED BY 5 EQUAL PERIODS IN SEA WATER CONTAINING 1 PER CENT ETHYL ALCOHOL

Solution	Change in PH	Relative rate of respiration
Sea water.....	$7.65-7.40=0.25$
" ".....	$7.65-7.35=0.30$
Sea water containing 1 per cent ethyl alcohol.....	$7.65-7.35=0.30$	$0.30 \div 0.275 = 1.1$
Sea water containing 1 per cent ethyl alcohol.....	$7.65-7.35=0.30$	$0.30 \div 0.275 = 1.1$
Sea water containing 1 per cent ethyl alcohol.....	$7.65-7.35=0.30$	$0.30 \div 0.275 = 1.1$
Sea water containing 1 per cent ethyl alcohol.....	$7.65-7.40=0.25$	$0.25 \div 0.275 = 0.91$
Sea water containing 1 per cent ethyl alcohol.....	$7.65-7.43=0.22$	$0.22 \div 0.275 = 0.80$

TABLE IV D, E, F CONTROL

SIX PERIODS (35.5 MIN. EACH) IN SEA WATER

Period	Change in PH	Relative rate of respiration
1.....	$7.65-7.45=0.20$	$0.20 \div 0.20 = 1.00$
2.....	$7.65-7.45=0.20$	$0.20 \div 0.20 = 1.00$
3.....	$7.65-7.45=0.20$	$0.20 \div 0.20 = 1.00$
4.....	$7.65-7.43=0.22$	$0.22 \div 0.20 = 1.10$
5.....	$7.65-7.43=0.22$	$0.22 \div 0.20 = 1.10$
6.....	$7.65-7.43=0.22$	$0.22 \div 0.20 = 1.10$

color of the indicator at the end of the second period of exposure was almost completely reversible, but after each subsequent period the color was fully reversible.

The reason for these instances of irreversibility can easily be explained. Phenolsulphonephthalein in the acid end of its range is yellow. *Laminaria* has a yellowish brown pigment which may come out when the cells are injured by very strong concentrations of the anesthetic, while the green pigment does not come out. When the concentration of the anesthetic is so great as to rapidly injure the cells (as is the case with all of the exceptions just noted),

the yellowish pigment comes out so rapidly as to interfere with the indicator. As soon as the extraction of the pigment ceases (usually ending during the first 2 periods), the color of the indicator is not interfered with. When the concentrations of anesthetics are lower than in those instances just mentioned, the pigment comes out (if at all) so slowly as not to affect the indicator. This can be proven by matching the solution obtained after any period (without the addition of indicator) with the color of sea water (containing no indicator) in a tube of equal diameter. In such cases no pigment is detectable.

Further evidence that it is CO_2 that is being measured in the experiments rather than other acids is the fact that by the use of the gas chain it was found that *Laminaria*, after being 2 weeks in a small quantity of unchanged sea water, had given off no acid other than CO_2 .

It might be supposed that the addition of so much alcohol as 24.2 per cent would dilute the buffer substances of the sea water so that a given amount of CO_2 added to the mixture would produce more change in PH value than would be the case in sea water. This was largely avoided by concentrating the sea water before adding the alcohol, so that the amount of buffer substance remained the same in the mixture as in the sea water alone. Tests made by adding measured amounts of CO_2 to sea water and to sea water plus 24.2 per cent alcohol (made up to the electrical conductivity of sea water) showed that there was not sufficient difference in this respect to be of importance in this investigation.

Such experiments enable us to follow the respiration of the same piece of tissue during shorter or longer periods of exposure to various concentrations of anesthetics. They further show that in no instance was there an initial decrease in the rate of respiration.

It will be observed that when the concentration of anesthetic is strong enough to produce any measurable result, the first effect is an increase of respiration, which gradually declines and may eventually fall below the normal. This decline is interpreted by the writer as a toxic effect.

These results are not in accord with the statements of TASHIRO and ADAMS (27), according to whom anesthetics do not produce

an increase of respiration except when their concentration is so low that they have only a stimulating action. They state that when the concentration is increased to the point where anesthesia occurs, the rate of respiration falls below the normal.

It is evident that this is not the case with *Laminaria*, for in no instance was the respiration observed to fall below the normal except after prolonged exposure to high concentrations which produced death. Further investigation will be necessary to determine the cause of these discrepancies.

It is evident that these experiments directly contradict the idea, advocated by VERWORN (29) and his pupils, that anesthesia is a kind of asphyxia and that anesthetics act by reducing respiration.

Summary

When *Laminaria* is exposed to anesthetics (in sufficiently high concentration to produce any result) the initial effect is an increase of respiration. This may be followed by a decrease if the anesthetic is sufficiently toxic. No decrease of respiration is observed when the concentration is too low to be toxic.

These results directly contradict the idea advocated by VERWORN and his pupils that anesthetics act by decreasing respiration.

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